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Characterisation of cefotaxime-resistant urinary *Escherichia coli* from primary care in South-West England 2017-2018

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Running heading: Cefotaxime-resistant urinary *E. coli*

Abstract

Objectives: Third-generation cephalosporin-resistant *Escherichia coli* from community-acquired urinary tract infections (UTI) are increasingly reported worldwide. We sought to determine and characterise the mechanisms of cefotaxime-resistance (CTX-R) employed by urinary *E. coli* obtained from primary care, over 12 months, in Bristol and surrounding counties in the South West of England.

Methods: Cephalexin resistant (Ceph-R) *E. coli* isolates were identified from general practice (GP) referred urine samples using disc susceptibility testing. CTX-R was determined by subsequent plating onto MIC breakpoint plates. β-Lactamase genes were detected by PCR. WGS was performed on 225 isolates and analyses were performed using the Centre for Genomic Epidemiology platform. Patient information provided by the referring GPs was reviewed.

Results: Ceph-R *E. coli* (n=900) were obtained from urines from 146 GPs. Seventy-percent (626/900) of isolates were CTX-R. WGS of 225 isolates identified that the most common CTX-R mechanism was $bla_{\text{CTX-M}}$ carriage (185/225), followed by plasmid mediated AmpCs (pAmpCs) (17/225), ESBL bla_{SHV} variants (6/225), AmpC-hyperproduction (13/225), or a combination of both $bla_{\text{CTX-M}}$ and pAmpC (4/225). Forty-four STs were identified with ST131 representing 101/225 isolates, within which clade C2 was dominant (54/101). Ciprofloxacin-resistance (CIP-R) was observed in 128/225 (56.9%) of sequenced isolates – predominantly associated with fluoroquinolone-resistant clones ST131 and ST1193.

Conclusions: Most Ceph-R *E. coli*'s were CTX-R, predominantly caused by $bla_{\text{CTX-M}}$ carriage. The correlation between CTX-R and CIP-R was largely attributable to the high-risk pandemic clones, ST131 and ST1193. Localised epidemiological data provides greater resolution than regional data and can be valuable for informing treatment choices in the primary care setting.

Introduction

Escherichia coli that are resistant to β-lactam antibiotics, particularly to cephalosporins, represent a major global public health concern. Third-generation cephalosporins (3GCs) are used across the world to treat infections caused by *E. coli* (e.g. urinary tract [UTIs], bloodstream [BSIs] and intra-abdominal infections) and subsequently the emergence of resistance is particularly worrying.[1] Resistance to 3GCs in *E. coli* can be caused by multiple mechanisms including chromosomally encoded AmpC β-lactamase hyperproduction, and may involve increased efflux, and reduced outer membrane permeability, but is predominantly attributed to the spread of plasmid-mediated ESBLs, (e.g. $bla_{CTX\text{-}M}$) and to a lesser extent, plasmid mediated AmpCs (pAmpC, e.g. bla_{CMY}).[2] *E. coli* which harbour ESBLs are often co-resistant to multiple antibiotic classes and subsequently the treatment options for such infections may be limited.[3]

UTIs are the most common bacterial infection type in both primary and hospital care settings in the developed world,[4] and are associated with considerable morbidity.[5] Previous studies of community-onset UTIs (CO-UTI) in several mainland European countries found that *E. coli* were the most commonly isolated uropathogen, accounting for over half of all isolates (53.3-76.7%).[6-8] The incidence of CO-UTI in the UK is difficult to determine since such infections are not reportable and most are diagnosed and treated in a primary care setting, with diagnosis often based solely upon patient symptoms rather than a positive urine culture. CO-UTI are most often treated empirically and subsequently local epidemiological data is useful for informing treatment choice. Treatment failure for CO-UTIs, particularly in immune-compromised patients, increases the risk of the infection spreading to other sites including the bloodstream, with grave consequences.[9, 10]

E. coli STs belonging to phylogroups B2 (STs 73, 95 and 131) and D (ST69) have been reported to be major causes of both UTIs and BSIs in the UK.[11] Since its initial description in 2008, numerous studies have shown that the multidrug-resistant pandemic clone, ST131, is a major cause of UTI globally.[12-14] The ST131 clonal group can be broken down by population

genetics into three clades based on their association with particular *fimH* types: A/*fimH*41, B/*fimH*22, and C/*fimH*30.[15] Clade C can be further broken down into four subclades; C1 – not usually associated with ESBL carriage but typically fluoroquinolone resistant (FQ-R), C1-M27 and C1-nM27 – both associated with $bla_{\text{CTX-M-27}}$ carriage and FQ-R, and C2 (also known as H30Rx) – associated with $bla_{\text{CTX-M-15}}$ carriage and FQ-R.[15, 16] Studies have suggested that the global dominance of ESBL-positive ST131 is, in part, due to its increased virulence potential over its non-ST131 ESBL-positive counterparts.[17, 18]

Although cephalosporin use for the treatment of UTIs is not typical in the UK, they may be used where resistance to first and second-line treatment exists. This may select cephalosporin resistant UTIs, and if an invasive infection occurs, empiric therapy with a 3GC is likely to fail. This study sought to use WGS to characterise the population structure and determine the mechanisms of resistance to the 3GC cefotaxime (CTX-R) employed by urinary *E. coli* isolates referred from general practices (GPs) in Bristol and surrounding counties in the South-West of England serving a population of approximately 1.2 million people.

Materials and Methods

Bacterial isolates, identification and susceptibility testing

Cephalexin-resistant (Ceph-R) urinary *E. coli* isolates were obtained from routine urine microbiology at Severn Infection Partnership Southmead Hospital. Urine samples were submitted between Sept 2017 and Aug 2018, from 146 GPs located throughout Bristol and including coverage in Gloucestershire, Somerset and Wiltshire.

Bacterial identification was carried out using BD™ CHROMagar™ Orientation Medium chromogenic agar (BD, GmbH, Heidelberg, Germany).

Antibiotic susceptibilities were performed by disc testing or, in the case of colistin, by broth microdilution and interpreted according to EUCAST guidelines.[19] A single colony from Ceph-

R isolates was subcultured onto TBX agar plates (Sigma-Aldrich, Dorset, UK) containing 2 mg/L CTX and isolates that were positive for growth were deemed CTX-R, and taken forward for further molecular testing.

Screening for β-lactamase genes

Two multiplex PCRs were performed to screen for β-lactamase genes. The first to detect $bla_{CTX\text{-}M}$ groups as previously described,[20] and the second to detect the following additional β-lactamase genes; $bla_{CMY\text{-}2}$ type, bla_{DHA}, bla_{SHV}, bla_{TEM}, $bla_{OXA\text{-}1}$, using the primers listed in Table S1.

WGS and analyses

WGS was performed by MicrobesNG (https://microbesng.uk/) on a HiSeq 2500 instrument (Illumina, San Diego, CA, USA) using 2x250 bp paired end reads. Reads were trimmed using Trimmomatic,[21] assembled into contigs using SPAdes[22] 3.13.0 (http://cab.spbu.ru/software/spades/) and contigs were annotated using Prokka.[23] Resistance genes, plasmid replicon types, sequence types and *fim* types were assigned using the ResFinder,[24] PlasmidFinder,[25] MLST[26] 2.0 and FimTyper on the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/) platform.

ST131 clades were identified by resistance gene carriage and *fimH* type, and in the case of clade C1-M27, by the presence of the prophage region M27PP1 (CP006632)[16] through sequence alignment using progressive Mauve alignment software.[27]

MLST and resistance gene data were analysed to produce a minimum spanning tree using Bionumerics software v7.6 (Applied Maths, Sint-Martens-Latem, Belgium).

Plasmid pUB_DHA-1 assembled onto a single contig. The overlap of contig ends was confirmed by PCR, and the plasmid sequence was submitted to GenBank with accession number MK048477. Reads were mapped using Geneious Prime 2019.1.3 (https://www.geneious.com).

Plasmid Transformation

Transformation of plasmid extractions from isolates encoding *mcr-1* and *bla*$_{\text{OXA-244}}$ were attempted by electroporation using *E. coli* DH5 alpha as a recipient. Transformants were selected, respectively, on LB agar containing 0.5 mg/L colistin, or containing 100 mg/L ampicillin with a 10 µg ertapenem disc being placed on the agar surface (Oxoid Ltd, Basingstoke, UK). Transformants were confirmed by PCR (Table S1).

Analysis of patient demographic information

Limited, non-identifiable patient information was obtained from the request forms sent with submissions from referring GPs.

Results and Discussion

Patient demographics and antimicrobial susceptibilities

E. coli is cultured from approximately two-thirds of all bacterium-positive GP-submitted urine samples processed by the Southmead Hospital laboratory, totalling ~36,000 isolates per year . Trimethoprim and nitrofurantoin resistance is observed in ~35% and 2%, and Ceph-R in ~8% of these *E. coli*, respectively. If the *E. coli* is Ceph-R, trimethoprim and nitrofurantoin resistance rates increase to ~67% and ~7%, respectively. 3GC resistance is seen in ~72% of Ceph-R *E. coli*.

Nine hundred Ceph-R urinary *E. coli* isolates were collected during the period of our study. Following deduplication by patient, isolates were obtained from 836 patients. Most isolates were obtained from female patients (669/836; 80.0%) and the mean patient age was 62.4 years (median = 69 years). Almost sixty-nine percent (576/836) were CTX-R, which matches phenotypic laboratory data, confirming that this is a representative sample. Most CTX-R isolates were again from females (465/576; 80.7%) and the mean patient age was 62.5 years (median = 69 years).

β-Lactamase genes of interest detected by PCR in CTX-R isolates

Table 1 indicates the number of CTX-R isolates carrying each β-lactamase gene of interest (GOI): *bla*$_{\text{CTX-Ms}}$, *bla*$_{\text{CMY}}$, *bla*$_{\text{DHA}}$ or *bla*$_{\text{SHV}}$. Of these, *bla*$_{\text{CTX-Ms}}$ were by far the most prevalent, found in 571/626 (91.2%) of isolates. Within these, *bla*$_{\text{CTX-M-G1}}$ were most common (421/626) followed by *bla*$_{\text{CTX-M-G9}}$ (149/626) and *bla*$_{\text{CTX-M-G8}}$ (one isolate). A previous study performed across four primary care trusts in England in 2013-14 estimated the carriage of CTX-M-producing Enterobacteriaceae in the faeces of healthy adults at 7.3% (range of 4.9-16.0%), over 95% of which were *E. coli*.[28] Based on our data above, 5.3% of GP-submitted urinary *E. coli* were CTX-M positive.

pAmpCs *bla*$_{\text{CMY}}$ and *bla*$_{\text{DHA}}$ were found in 13 (3 alongside *bla*$_{\text{CTX-M-G1}}$) and 17 (4 alongside *bla*$_{\text{CTX-M-G1}}$) isolates respectively. *bla*$_{\text{SHV}}$ was found in 11 (3 alongside *bla*$_{\text{CTX-M-G1}}$) isolates and the remaining 24 isolates harboured none of the GOIs as detected by PCR.

Whole Genome Sequencing (WGS) analyses

GOI variants and STs

Two-hundred and twenty-five isolates, chosen to be representative of resistance gene carriage (as previously determined by PCR) and patient demographics (age, sex) obtained throughout the entire study period, were selected for WGS. Within these, 44 sequence types (STs) were identified, with numbers of isolates ranging from 1 to 101 representatives. ST131 was dominant (n=101), followed by STs 69 (n=15), 73 (n=15), 38 (n=13), 1193 (n=11), and 10 (n=8). The remaining 38 STs had 1 to 4 representative isolates (Figure 1).

CTX-R GOIs were identified in all but thirteen isolates (212/225; 94.2%). Eighty-four percent (189/225) of isolates harboured one of seven *bla*$_{\text{CTX-M}}$ gene variants (Table 2). Carriage of *bla*$_{\text{CTX-M-15}}$ was the most common CTX-R mechanism identified (118/189) followed by *bla*$_{\text{CTX-M-27}}$ (44/189) and *bla*$_{\text{CTX-M-14}}$ (10/189). Amongst the non-CTX-M GOIs, four *bla*$_{\text{CMY}}$ variants were

identified; *bla*$_{CMY-2}$ (n=7), *bla*$_{CMY-4}$ (n=1), *bla*$_{CMY-42}$ (n=2) and *bla*$_{CMY-60}$ (n=3; all three being co-carried alongside *bla*$_{CTX-M-15}$), as well as *bla*$_{DHA-1}$ (n=8; one alongside *bla*$_{CTX-M-15}$) and *bla*$_{SHV-12}$ (n=6). The narrow spectrum β-lactamases *bla*$_{OXA-1}$, *bla*$_{TEM-1}$ and inhibitor-resistant variant *bla*$_{TEM-33}$ were found in 53, 82, and one isolate respectively.

AmpC-hyperproducing isolates

All thirteen (5.8%) sequenced isolates, where no CTX-R GOI could be identified, were presumed to be chromosomal AmpC β-lactamase hyperproducers because they carry mutations within the *ampC* promoter/attenuator region previously seen in confirmed AmpC hyperproducers (Table 3).[29, 30] These represented nine different STs, each having one representative, with the exceptions of the STs 75 and 200 of which there were three representatives each (Table S2). This indicates a lack of dominant clones in AmpC hyperproducers identified in this study. If we go on to assume that the 24 isolates negative for GOIs by PCR are AmpC-hyperproducers, as was found with the thirteen representative sequenced isolates, then 3.8% of the isolates in this study could be classed as AmpC-hyperproducers. Additionally, one *bla*$_{CTX-M-15}$-positive isolate was found to also harbour *ampC* promoter changes associated with hyperproduction.

Characterisation of ST131 Isolates

ESBLs and clades

One hundred and one ST131 isolates harboured the following CTX-R mechanisms/alleles; *bla*$_{CTX-M-1}$ (n=1), *bla*$_{CTX-M-14}$ (n=4), *bla*$_{CTX-M-15}$ (n=61), *bla*$_{CTX-M-15}$/*bla*$_{CMY-60}$ (n=1), *bla*$_{CTX-M-27}$ (n=33). The isolates were broken down into their respective clades (Table 4); ST131-C2 was dominant (54/101; 53.5%) followed by C1-M27 (M27) (23/101; 22.8%), A (11/101; 10.9%), C1-nM27 (5/101; 5.0%), one clade B, and seven isolates were unclassified. Eighty-eight percent (89/101) of isolates, and notably all clade C2 isolates, were CIP-R as is a typical characteristic of this lineage. Two non-ST131 members of the ST131 complex, both of which were *bla*$_{CTX-M-15}$-positive ST8313 isolates (a *fumC* single locus variant (SLV) of ST131), also harboured the

same chromosomal FQ-R associated mutations in *gyrA*, *parC* and *parE* as are associated with ST131/C2 – suggesting this ST may be ST131/C2 derived. Previous studies have highlighted the dominance of ST131 and particularly the clade C2/H30Rx on a worldwide scale.[13, 14] Since its initial description in 2008 in isolates from 3 continents,[12, 13] ST131 has been reported across all inhabited continents.[15] The recently described C1 subclade, C1-M27, as characterised by the presence of a 11,894 bp prophage-like genomic island M27PP1, was initially described in Japan in 2016 and has been reported in countries in at least three continents: Europe, Asia and North America, so far.[16] The presence of C1-M27 isolates in this study indicates the expansion of this particular ST131 sublineage into the UK, similarly to that which has been reported from countries in mainland Europe.[31] There was no geographical clustering of C1-M27 positive isolates within our study region.

Virotypes

ST131 has been reported in previous studies to be a highly virulent clone, exhibiting lethality in mouse sepsis models.[18, 32] Virotypes of all 101 ST131 isolates were determined as previously described.[18, 33] Virotype C was most common, represented by 38/101 (37.6%) of ST131 isolates and predominantly associated with *bla*$_{\text{CTX-M-27}}$ (28/38 isolates) across clades A (n=3), C1-M27 (n=22), C1-nM27 (n=4), and one isolate belonged to an unclassified clade. All *bla*$_{\text{CTX-M-27}}$-positive isolates belonged to virotype C, with the exception of one isolate for which a virotype could not be assigned. The association between virotype C and *bla*$_{\text{CTX-M-27}}$ carriage is in agreement with a recent study conducted in France.[34] Twenty-five isolates belonged to virotype A, all of which except one harboured *bla*$_{\text{CTX-M-15}}$. The remaining 38 isolates belonged to either virotype B (n=1), D (n=1), G (n=8), or were unknown virotypes (n=28).

Genetic context of ESBLs/pAmpC genes

Despite the limitations of short read sequencing, by examining the contigs on which GOIs were located, we were able to determine the chromosomal or plasmid environments of ESBLs/pAmpC genes in 85/225 isolates. Forty *bla*$_{\text{CTX-M}}$ genes, of variants *bla*$_{\text{CTX-M-14}}$ (n=3) and

bla$_{CTX-M-15}$ (n=37), were found to be located on the chromosome. These were found in 11 STs with STs 131 (n=11) and 73 (n=10) being the most represented. Whilst the *bla*$_{CTX-M}$ genetic environments were diverse in ST131, in ST73, 9/10 isolates harboured the gene in the same genomic location, suggesting a high degree of clonality within this ST. Chromosomally encoded *bla*$_{CTX-M}$ genes have previously been reported in 12.9% of human isolates in study of ESBL *E. coli* performed in Germany, The Netherlands and the UK.[35] The remaining GOIs were found to be located within a relatively diverse range of plasmids and across multiple STs. Interestingly all six DHA-1-harbouring isolates were found to harbour a similar IncI1 plasmid which was sequenced to closure during this study, pUB_DHA-1. Read mapping analyses showed that all six isolates exhibited 95-100% coverage and 98-100% identity against pUB_DHA-1.

Other important resistance genes found by WGS

Interestingly one ST69 CMY-2-producing isolate was also found to harbour *mcr-1*. Susceptibility testing, performed by broth microdilution, revealed that the MIC of colistin against this isolate is 8 mg/L and so it is colistin resistant. Attempts at transformation of *E. coli* DH5 alpha using plasmid DNA extracted from this isolate were successful indicating that *mcr-1* is plasmid encoded. Analysis of the genetic environment of *mcr-1* found that it is encoded on an IncI2 plasmid of approximately 62 kb and it lacks the upstream IS*Apl1* element that was described in the initial discovery of *mcr-1* in China.[36] The plasmid itself does not encode any additional resistance genes and when subjected to NCBI BLAST analysis exhibited ~96% similarity to *mcr-1* encoding plasmids found in both China (pHNGDF93; Genbank Accession Number MF978388) and Taiwan (p5CRE51-MCR-1; Genbank Accession Number CP021176) from animal and human origins, respectively. Since initial reports of its discovery in 2015,[36] *mcr-1* has been reported worldwide in clinical *E. coli* isolates although remains relatively rare.

Another isolate, a *bla*$_{CTX-M-14}$-positive ST38, also encoded the *bla*$_{OXA-48}$-like carbapenemase gene *bla*$_{OXA-244}$.[37] Transformation attempts using plasmid DNA from this isolate were unsuccessful and so it was concluded that *bla*$_{OXA-244}$ is likely to be chromosomally encoded.

Disc susceptibility testing showed that this isolate was resistant to ertapenem but susceptible to both imipenem and meropenem. The presence of the chromosomally-encoded OXA-48-like carbapenemase *bla*$_{OXA-244,}$ confirms the observations of a previous study, where OXA-48-like genes were shown to have become embedded in the ST38 chromosome.[38] ST38 is the most frequent ST associated with OXA-48-like enzymes in the UK.[38]

Conclusions

Resistance to 3GCs and FQs in *E. coli* is of increasing concern due to the importance of these classes of drugs for the treatment of serious infections. Increasing resistance to 3GCs puts increased pressure and reliance on carbapenems, often referred to as the 'antibiotics of last resort' for serious MDR Gram-negative infections and subsequently the surveillance of these resistances is essential. As observed in this study and in line with previous reports globally,[2] the dissemination of successful clones, and/or ST lineages (clades), is a major cause of CTX-R in urinary isolates from primary care in South West England.

bla$_{CTX-M}$-positive *E. coli* have been observed in our study region since initial reports in 2000.[39] The prevalence of *bla*$_{CTX-M}$ genes observed in the CTX-R isolates in this study is reflective of the observed rates of CTX-M *E. coli* faecal carriage in healthy adults in the UK, as has been quantified in previous studies.[28, 40]

The correlation between CIP-R and CTX-R highlighted here can be largely attributed to the dominance of successful clones/clades, namely ST131 and ST1193; the majority of both harbour chromosomal FQ-R mutations. Through WGS of a subset of isolates we have shown that ST131 clade C2 is dominant and that the recently described ST131 subclade, C1-M27, is also prevalent despite not previously being described in the UK.

This study is the first analysis of CTX-R *E. coli* causing CO-UTI performed in a relatively localised area in South West England and could be useful for informing patient treatment, alongside resistance information generated in the hospital laboratory, to provide essential data for comparison purposes to other areas, both within and outwith the UK.

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Transparency declaration

None to declare.

Supplementary data

Tables S1 and S2 are available as supplementary data.

References

1. Public Health England. English surveillance programme for antimicrobial utilisation and resistance (ESPAUR). 2018. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/759975/ESPAUR_2018_report.pdf.
2. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M beta-lactamases: temporal and geographical shifts in genotype. *J Antimicrob Chemother* 2017; **72**: 2145-55.
3. Morosini MI, Garcia-Castillo M, Coque TM *et al*. Antibiotic coresistance in extended-spectrum-beta-lactamase-producing Enterobacteriaceae and in vitro activity of tigecycline. *Antimicrob Agents Chemother* 2006; **50**: 2695-9.
4. Nicolle LE. Epidemiology of urinary tract infections. *Clin Microb News* 2002; **24**: 135-40.
5. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med* 2002; **113 Suppl 1A**: 5s-13s.
6. Kahlmeter G, Eco.Sens. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS Project. *J Antimicrob Chemother* 2003; **51**: 69-76.
7. Cordoba G, Holm A, Hansen F *et al*. Prevalence of antimicrobial resistant *Escherichia coli* from patients with suspected urinary tract infection in primary care, Denmark. *BMC Infect Dis* 2017; **17**: 670.
8. Schito GC, Naber KG, Botto H *et al*. The ARESC study: an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. *Int J Antimicrob Agents* 2009; **34**: 407-13.
9. Song JAW, Berridge D, Akbari A, *et al*. Risk factors for *Escherichia coli* bacteraemia: a population-based case-control study. *Lancet; Meeting Abstracts* 2017; **390:** S85.
10. Lishman H, Costelloe C, Hopkins S *et al*. Exploring the relationship between primary care antibiotic prescribing for urinary tract infections, *Escherichia coli* bacteraemia incidence and antimicrobial resistance: an ecological study. *Int J Antimicrob Agents* 2018; **52**: 790-8.
11. Doumith M, Day M, Ciesielczuk H *et al*. Rapid identification of major *Escherichia coli* sequence types causing urinary tract and bloodstream infections. *J Clin Microbiol* 2015; **53**: 160-6.
12. Coque TM, Novais A, Carattoli A *et al*. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. *Emerg Infect Dis* 2008; **14**: 195-200.
13. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V *et al*. Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. *J Antimicrob Chemother* 2008; **61**: 273-81.
14. Petty NK, Ben Zakour NL, Stanton-Cook M *et al*. Global dissemination of a multidrug resistant *Escherichia coli* clone. *Proc Nat Acad Sci USA* 2014; **111**: 5694-9.
15. Pitout JD, DeVinney R. Escherichia coli ST131: a multidrug-resistant clone primed for global domination. *F1000 Res* 2017; **6**.
16. Matsumura Y, Pitout JD, Gomi R *et al*. Global *Escherichia coli* Sequence Type 131 Clade with blaCTX-M-27 Gene. *Emerg Infect Dis* 2016; **22**: 1900-7.
17. Banerjee R, Robicsek A, Kuskowski MA *et al*. Molecular epidemiology of *Escherichia coli* sequence type 131 and Its H30 and H30-Rx subclones among extended-spectrum-beta-lactamase-positive and -negative *E. coli* clinical isolates from the Chicago Region, 2007 to 2010. *Antimicrob Agents Chemother* 2013; **57**: 6385-8.
18. Mora A, Dahbi G, Lopez C *et al*. Virulence patterns in a murine sepsis model of ST131 *Escherichia coli* clinical isolates belonging to serotypes O25b:H4 and O16:H5 are associated to specific virotypes. *PloS One* 2014; **9**: e87025.
19. European Committee of Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.1.

http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.1_Breakpoint_Tables.pdf.
20. Woodford N, Fagan EJ, Ellington MJ. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum (beta)-lactamases. *J Antimicrob Chemother* 2006; **57**: 154-5.
21. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014; **30**: 2114-20.
22. Bankevich A, Nurk S, Antipov D *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comp Biol* 2012; **19**: 455-77.
23. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014; **30**: 2068-9.
24. Zankari E, Hasman H, Cosentino S *et al*. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 2012; **67**: 2640-4.
25. Carattoli A, Zankari E, Garcia-Fernandez A *et al*. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014; **58**: 3895-903.
26. Wirth T, Falush D, Lan R *et al*. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol* 2006; **60**: 1136-51.
27. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PloS One* 2010; **5**: e11147.
28. McNulty CAM, Lecky DM, Xu-McCrae L *et al*. CTX-M ESBL-producing Enterobacteriaceae: estimated prevalence in adults in England in 2014. *J Antimicrob Chemother* 2018; **73**: 1368-88.
29. Caroff N, Espaze E, Gautreau D *et al*. Analysis of the effects of -42 and -32 ampC promoter mutations in clinical isolates of *Escherichia coli* hyperproducing ampC. *J Antimicrob Chemother* 2000; **45**: 783-8.
30. Peter-Getzlaff S, Polsfuss S, Poledica M *et al*. Detection of AmpC beta-lactamase in *Escherichia coli*: comparison of three phenotypic confirmation assays and genetic analysis. *J Clin Microbiol* 2011; **49**: 2924-32.
31. Merino I, Hernandez-Garcia M, Turrientes MC *et al*. Emergence of ESBL-producing *Escherichia coli* ST131-C1-M27 clade colonizing patients in Europe. *J Antimicrob Chemother* 2018; **73**: 2973-80.
32. Clermont O, Lavollay M, Vimont S *et al*. The CTX-M-15-producing *Escherichia coli* diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. *J Antimicrob Chemother* 2008; **61**: 1024-8.
33. Barrios-Villa E, Cortes-Cortes G, Lozano-Zarain P *et al*. Adherent/invasive *Escherichia coli* (AIEC) isolates from asymptomatic people: new *E. coli* ST131 O25:H4/H30-Rx virotypes. *Ann Clin Microbiol Antimicrob* 2018; **17**: 42.
34. Birgy A, Levy C, Nicolas-Chanoine MH *et al*. Emergence and dominance of *E. coli* ST131 CTX-M-27 in a community paediatric cohort study: independent host factors and bacterial genetic determinants. *Antimicrob Agents Chemother* 2019; 63:e00382-19.
35. Rodriguez I, Thomas K, Van Essen A *et al*. Chromosomal location of bla_{CTX-M} genes in clinical isolates of *Escherichia* coli from Germany, The Netherlands and the UK. *Int J Antimicrob Agents* 2014; **43**: 553-7.
36. Liu YY, Wang Y, Walsh TR *et al*. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* 2016; **16**: 161-8.
37. Oteo J, Hernandez JM, Espasa M *et al*. Emergence of OXA-48-producing *Klebsiella pneumoniae* and the novel carbapenemases OXA-244 and OXA-245 in Spain. *J Antimicrob Chemother* 2013; **68**: 317-21.
38. Turton JF, Doumith M, Hopkins KL *et al*. Clonal expansion of *Escherichia coli* ST38 carrying a chromosomally integrated OXA-48 carbapenemase gene. *J Med Microbiol* 2016; **65**: 538-46.

396 39. Tarrant F, MacGowan AP, Walsh TR. Occurrence and current frequency of CTX-M-type beta-
397 lactamases from a regional hospital in the South West of England. *J Antimicrob Chemother* 2007; **59**:
398 815-6.
399 40. Wickramasinghe NH, Xu L, Eustace A *et al*. High community faecal carriage rates of CTX-M
400 ESBL-producing *Escherichia coli* in a specific population group in Birmingham, UK. *J Antimicrob*
401 *Chemother* 2012; **67**: 1108-13.

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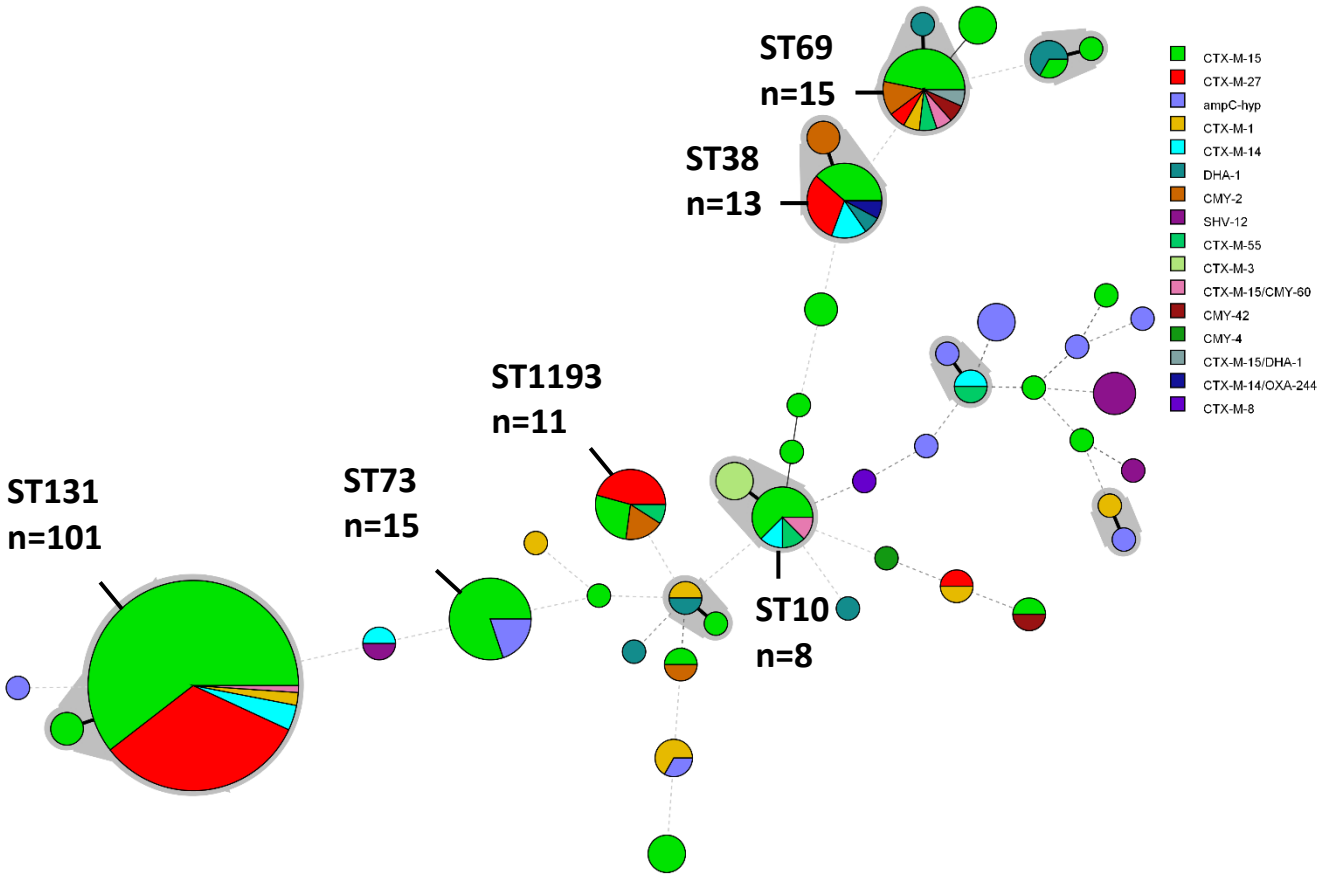

Figure 1. Minimum spanning tree of the MLST profiles of 225 CTX-R *E. coli* isolates. The shaded areas represent single locus variants (SLVs). Members of the most prevalent STs (>4 representatives) are labelled and their number of representatives indicated. The diameter of the circle represents the number of isolates of that particular ST and the coloured segments indicate which CTX-R mechanisms were identified. Thick solid lines represent SLVs, thin solid lines represent double-locus variants and dashed connecting lines indicate multilocus variants. This figure appears in colour in the online version of JAC and in black and white in the printed version of JAC.

	Isolates (M/F)	CTX-R mechanisms identified by PCR									
		CTX-M G1	CTX-M G1 + DHA	CTX-M G1 + SHV	CTX-M G1 + CMY	CTX-M G9	CTX-M G8	CMY	DHA	SHV	None
	F (n=507)	334	3	1	2	123	1	6	11	6	20
	M (n=119)	77	1	2	1	26	0	4	2	2	4
Total	**626**	**411**	**4**	**3**	**3**	**149**	**1**	**10**	**13**	**8**	**24**

Table 1. Beta-lactamase genes detected by multiplex PCRs on 626 CTX-R isolates.

bla$_{CTX-M}$ variant							*bla*$_{CMY}$ variant				*bla*$_{DHA}$ variant	*bla*$_{SHV}$ variant
CTX-M-1	CTX-M-3	CTX-M-8	CTX-M-14	CTX-M-15	CTX-M-27	CTX-M-55	CMY-2	CMY-4	CMY-42	CMY-60	DHA-1	SHV-12
9	**3**	**1**	**10**	**118**	**44**	**4**	**7**	**1**	**2**	**3**	**8**	**6**

Table 2. ESBL/pAmpC variants identified in the 225 isolates subjected to WGS.

Note: 3 isolates harboured both *bla*$_{CMY-60}$ and *bla*$_{CTX-M-15}$, and one isolate harboured *bla*$_{DHA-1}$ and *bla*$_{CTX-M-15}$.

No. of isolates	*ampC* promoter/attenuator mutations	Pribnow box
8	-42C>T, -25G>A, -1C>T, +57C>T	TTGACA - 17nt - TATCGT
2	-28G>A, ins -12 T -13, +22G>T	TTGTCA - 17nt - TACAAT
2	-11C>T, ins -12 T -13, +33G>A, +36G>A	TTGTCA - 17nt - TATAAT
1	-32T>A, +34C>A, +57C>T	TTGACA - 16nt - TACAAT

Table 3. Mutations found within promoter/attenuator region of the 13 presumed AmpC-hyperproducing isolates subjected to WGS relative to *E. coli* MG1655 (Genbank Accession Number NC_000913.3).

ST131 Clades (no.)			CTX-R Alleles				
			CTX-M-1	CTX-M-14	CTX-M-15	CTX-M-27	CMY-60
A		11	1	1	4	5	
B		1	1				
C	C1-M27	23				23	
	C1-nM27	5		3		2	
	C2	54			54		
Unclassified		7			4[a]	3	1[a]
Total		101	2	4	62	33	1[a]

Table 4. ST131 clades and CTX-R GOI alleles harboured by 101 isolates subject to WGS.

[a] One isolate belonging to an ST131 unclassified clade harboured both *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{CMY-60}}$.